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NUTRITION REVIEWS

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NUTRITION REVIEWS

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VITAMIN E

None of the vitamins has been associated with as wide a variety of biologic processes as vitamin E, or has been functionally related to so many basically different physiologic and chemical reactions. The name tocopherol, an alcohol concerned in the bearing of young, connotes only one of the vital roles which it plays. Its importance in neuromuscular metabolism was foreshadowed many years before it was isolated in purity and given a chemical name, and the departures from the normal behavior and composition of tissues produced by lack of it include a baffling array of unexplained phenomena.

The manner in which it participates in the process of reproduction is not understood. The responses to its absence appear to differ fundamentally in male and female rats, and the need of it in other species has been definitely established in only a few instances. Two reasons may be assigned for this uncertainty. Appropriate diets adequate in all other respects over long periods of time are difficult to formulate. Furthermore, such diets, when otherwise complete, produce prior disabilities which may prove fatal before the animals become sexually mature. Thus, young rabbits and guinea pigs succumb to nutritional muscular dystrophy in a few weeks. So do calves and lambs and ducklings.

Laying hens, when given a ration lacking vitamin E, produce eggs of low hatchability; when normal day-old chicks are placed on vitamin E-deficient feed, they develop exudative diathesis or encephalomalacia, depending on the character of the diet in respect to its other components, such as protein, fat and minerals.

Not only is there a wide diversity in the manifestation of vitamin E deficiency in different species, but the results are not "absolute." They also exhibit a disconcerting variability in respect to the age of the experimental animal quite apart from the matter of storage. Thus, chicks may recover spontaneously, and do so, under field conditions. Nursling rats may recover from their paralysis without any change in regimen and thereafter progress normally and arrive at sexual maturity before their inherent disability is recognized, whereas in the case of the young growing rabbit a fraction of a milligram of tocopherol, daily, stands between him and death.

But if the rat continues to have no access to tocopherol, the incisors become depigmented and there appears in many tissues an inert brownish pigment, derived perhaps from fat, perhaps from protein. The animal exhibits a progressively increasing paralysis and ends its days in miserable helplessness. If, to these almost paradoxic observations on the role of vitamin E one adds protection against liver necrosis on low-protein diets, and prevention of the increased red-cell fragility and hemolysis produced by alloxan-like substances, the list becomes truly formidable.

The search for some unifying principle or correlating idea as to the manner of action has not been rewarding. An early suggestion emerged from the fact that tocopherol is the principal antioxygenic component of natural fats. Its rapid disappearance in autoxidizing fats, by themselves and in food mixtures, was, indeed, the occasion of its discovery. The progress of its oxidation has been fairly well documented, through a free radical form and an epoxide to the quinone. The quinone and the unstable hydroquinone have biopotency in rabbits and perhaps in human muscle disorders, but

not in the rat. If the role of tocopherol involves reversible oxidation and reduction, the conditions seem to vary from one species to another or from one function to another.

Perhaps the tocopherols as biologic antioxidants have a protective function in preventing the appearance of tissue-damaging products of oxidation, among them fat peroxides. The various kinds of radiation, cosmic and otherwise, to which animals are exposed, generate peroxidic substances and, like sulfhydryl groups in the aqueous phase, tocopherol, soluble in the fat phase, may be needed to destroy them, vicariously. This implies the notion of chain reactions which can be interrupted by very small amounts of chain-breakers, but the study of chain reactions and free radicals in living systems is in its infancy. The damage by peroxidic substances might explain the brown pigment as a polymer of unusable fat peroxides or of aberrant and nonmetabolizable products of protein degradation. Perhaps peroxides alter certain fundamental properties of cytoplasmic proteins to account for the vascular changes, as in exudative diathesis and embryonic death, or of the nuclear proteins leading to testicular degeneration and the dystrophic condition in muscle.

Presumably these disastrous detours from the normal metabolic highway are occasioned by the disorganization of some of the enzyme systems which normally direct the traffic. A study of the enzyme systems in dystrophic muscle tissue promises to be especially intriguing because a good deal is known about the progress of reactions in normal muscle and because the usual course of events is so profoundly disrupted in some animals when vitamin E is withheld. Most striking, perhaps, is the great increase in the rate of oxygen uptake by dystrophic muscle preparations *in vitro*, as compared with that of similar preparations from muscles of normal animals, together with notable changes in physiologic properties and in chemical composition, notably the loss of creatine and the accompanying creatinuria. Superficially, here is a real example of the action of a biologic antioxidant, but chemically and biochemically, the problem is in no wise simple. The alterations in enzyme concentrations, as measured individually, are not always consistent with the functional changes, and the addition of tocopherol to preparations *in vitro* does not restore normal conditions. But neither are the amounts and characteristics of the enzymes of normal tissue consistent from one species to another; nevertheless, their combined action eventuates in normal muscle metabolism and contraction. Furthermore, their concentrations change from youth to age.

In the chart of consecutive oxidative reactions in muscle tissue *in vitro*, as it is formulated in most textbooks, are the various steps characteristic of the active or the resting metabolism of muscle? Perhaps this is an academic question since the two can likely never be separated, but creatinine and creatine are not end-products of the metabolism of contraction; their excretion does not vary with muscular activity. For the ultimate explanation of the creatinuria and the loss of creatine from muscle in dystrophy, the presently accepted scheme of events is inadequate.

From this welter of factual information the explanation will some day emerge as to why a growing rabbit cannot live without vitamin E, whereas a growing rat can.

It is possible to produce muscle degeneration in a variety of ways and the resulting histologic picture is much the same. Some of the degenerative changes unhappily found in human muscle show the same structural alterations, but neither these nor the atrophy following nerve section can be reversed by the *in vivo* administration of vitamin E. Many more clues must be provided before the puzzles posed by vitamin E can be solved.

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RELATION OF MALNUTRITION TO FLUORIDE TOXICITY

Discussions on the possible relationship of malnutrition to the incidence of mottled enamel have been frequent and varied in their conclusions. C. D. M. Day (*Brit. Dent. J.* **68,** *409* (*1940*)) believed that his surveys in the Punjab province of Northern India indicated a higher incidence and a greater severity of mottling of the enamel among the malnourished inhabitants than among the well nourished individuals. Likewise, D. C. Wilson (*Lancet* **I,** *211, 375* (*1941*)) stated that in her experience mottled teeth in children whose nutriture was assessed as subnormal tended to be accompanied by a pitting and attrition of the enamel which was not evident in the well nourished children.

However, in a survey in North and South Shields in England, where the drinking water supplies contained 0.25 and 1.4 parts per million (p.p.m.) of fluorides, respectively, R. Weaver (*Brit. Dent. J.* **76,** *29* (*1944*)) found no evidence of a relation between malnutrition and mottled enamel. Later, in a survey of a chronic endemic fluorosis area of Morocco, M. M. Murray and D. C. Wilson (*Ibid.* **84,** *97* (*1948*)) concluded from studies of two groups of 25 children each, one European and the other Arab, that the Arab children had more severe mottling. The water supply was common to both groups, but the European children were judged to be much better nourished than the Arabian children.

M. Massler and I. Schour (*J. Am. Dent. Assn.* **44,** *156* (*1952*)) conducted a survey in Italy in 1945 to determine if there were any possible effects of malnutrition on the response of the developing enamel to waterborne fluorides. Two Italian communities were selected for this survey: Campagnano di Roma, a community of about 1000 inhabitants situated about 35 miles northwest of Rome near the peak of Mt. Rossi, and Quarto, a community of less than 1000 inhabitants lying in a valley about 15 miles from Naples, separated from the Mediterranean Sea by rocky hills.

The community of Campagnano di Roma was entirely agricultural and was well supplied with wheat, vegetables, and livestock. Throughout the war, the community was reasonably self-sufficient with respect to food. At the time of the survey, the inhabitants were moderately nourished on the average, but were not judged to be so well nourished as the average individual in most cities in the United States. Seventy-eight per cent of the adults were judged to be in a state of adequate nutriture and only 20 per cent were believed to be malnourished. However, only 32 per cent of the children, 6 to 14 years old, were found to be well nourished and 50 per cent were definitely malnourished.

The town of Quarto was entirely agricultural, but raised little besides apples. The economic status and the standard of living were low. Signs of malnutrition were apparent in nearly all the people. Evidence of frank rickets, dry scaly skin with multiple sores, angular cheilosis and red fissured tongues were found in almost every inhabitant by simple inspection. Only 1.5 per cent of the children, 6 to 14 years of age, were judged to be well nourished, 2.9 per cent were in a state of subnutrition, 39.7 per cent were mildly malnourished, 44.1 per cent were in a state of moderate malnutrition, and 11.8 per cent were in a condition of severe malnutrition. The major nutritional disturbances in both towns were rickets, deficiencies in the vitamin B complex, in protein and in calories.

The water supply was obtained from a village well in both cases. The fluoride content of the water in Campagnano di Roma was 3.5 p.p.m. and at Quarto was 1.3 p.p.m. The amount of mottling of the inhabitants' teeth was scored from 0 for no mottling present to V for severe mottling. The amount of mottling in the teeth of the

inhabitants of Campagnano di Roma was compared with the amount observed in Conway, South Carolina, where the fluoride content of the water is 4.0 p.p.m. The percentage of children with mottled teeth at Campagnano di Roma was 100 while the incidence in Conway was 88.2. In addition, the average extent or index of dental fluorosis was 2.52 for the children in Campagnano di Roma in contrast to 2.10 for the children of Conway. The amount of mottling of the teeth of citizens of Quarto was compared with the degree of fluorosis seen in Joliet, Illinois, where the water supply contains 1.3 p.p.m. of fluorides. The percentage of children with mottling in Quarto was 60.0, with only 25.3 per cent of Joliet children exhibiting any signs of abnormality. The index of dental fluorosis in Quarto was 1.20 in comparison with 0.46 for Joliet. The inhabitants of Conway, South Carolina, and Joliet, Illinois, were reported to be in a much better state of nutrition on the average than the residents of the two Italian communities.

The investigators commented in considerable detail on the differences in the amount of mottling between the inhabitants of the two American cities and those in the two Italian communities with comparable fluoride-bearing water supplies. They emphasized that the higher incidence of dental fluorosis among the Italian citizens could not be attributed to higher consumption of water; on the contrary, they felt that the water consumption in Campagnano di Roma and Quarto was appreciably less than in the United States towns. They believed that the only discernible reason for the increased prevalence and the increased severity of mottling was the fact that malnutrition was more severe in the Italian children and adults than in the United States. An observation which may strengthen this point is the higher incidence of moderately severe and severe mottling among the inhabitants of Quarto where the malnutrition was the most serious.

In an effort to study more exactly whether the degree of malnutrition might be correlated with the susceptibility to dental fluorosis, they determined the degree of association between understature and the extent of mottling and the degree of association between physical status as estimated by the Wetzel grid and the extent of mottling. In the first instance, the coefficient of association was 0.361, and in the second case it was 0.601, where perfect agreement would be 0.9 and no agreement would be zero. Thus, there appeared to be a tendency toward more mottling in the lower height-lower weight groups.

The investigators tended to implicate inadequate calcium nutriture as the important factor in the causation of the increased mottling rather than any of the other obvious deficiencies. This proposal was based largely on the fact that numerous studies with experimental animals have shown that calcium deficient animals were more susceptible to fluoride toxicity than their normal controls. The effects of other nutritional deficiencies on the toxicity of fluorides have not been studied in sufficient detail in experimental animals to permit evaluation of their potential relationships.

On the basis of present knowledge, the question of whether or not malnutrition influences the incidence and extent of dental fluorosis cannot be answered with any degree of certainty. Massler's and Schour's implication of calcium deficiency specifically seems to be premature, for a variety of other factors should first be considered in enough detail to be evaluated accurately. In the examination and evaluation of a survey such as the one described in this review, the reader cannot help asking questions about the constancy of the fluoride concentration in the water supply, and whether or not the fluoride concentration of the foodstuffs was checked. For example, in the community of Quarto where the chief crop was apples, was a fluoride-containing spray employed for insect control without adequate cleansing

of the apples before consumption? Again, were these communities sufficiently close to active volcanoes that volcanic ash containing fluorides might have been deposited on the vegetation of the gardens and farms in these areas? Likewise one must consider the question of water consumption which was dismissed as inconsequential because the investigators believed a low water consumption existed in these communities. Was the survey sufficiently detailed to determine this point with accuracy? Are the cooking procedures such in these Italian communities that more water is used in food preparation than in American towns, either because much water is needed to soak the uncooked foods, or because long cooking periods are used with extensive evaporation and hence concentration of the fluorides? None of these questions may be involved in the differences between the incidence and extent of tooth mottling in these Italian communities and towns in the United States with similar fluoride concentrations, but on the other hand, they cannot be casually dismissed as irrelevant.

NATURE AND MODE OF ACTION OF THE INTRINSIC FACTOR

Previous articles (*Nutrition Reviews* **3,** *7* (*1945*); **6,** *193* (*1948*); **7,** *146* (*1949*); **8,** *81*, *165* (*1950*); **9,** *281* (*1951*)) have summarized the evidence pointing to the fact that vitamin B_{12} may be both the extrinsic (food) and the maturation (liver) factor in the older scheme representing the mode of synthesis of the antipernicious anemia factor: intrinsic factor + extrinsic factor → erythrocyte maturation factor. This revision of the original concept no longer requires the formation of a different maturation factor, and implies that the physiologic role of intrinsic factor is actually either to promote the absorption or to prevent the destruction or competitive utilization within the intestine of the small amounts of vitamin B_{12} normally found in foods. Previous observations indicate that in untreated pernicious anemia, the hematopoietic activity of orally administered vitamin B_{12} is increased by the simultaneous administration of intrinsic factor, but never to the extent that it equals the activity of the same amount of vitamin B_{12} given alone parenterally.

The important new work of A. D. Welch, V. Scharf, R. W. Heinle, and G. C. Meacham (*Fed. Proc.* **11,** *308* (*1952*)) indicates that 70 to 95 per cent of the amount of radioactive cobalt given in the form of daily oral doses of 0.5 micrograms of isotopically labelled cobalt-containing vitamin B_{12} is excreted in the feces when given alone to patients with pernicious anemia in remission, but that only 5 to 30 per cent is excreted when given with intrinsic factor. Together, the old and the new clinical evidence thus leave little doubt that enhanced absorption of vitamin B_{12} is indeed the effect produced by intrinsic factor.

Although the clarity of this conclusion by no means illuminates the further obscurity surrounding what intrinsic factor is or how it works, several interesting experimental approaches to these questions have been made. Thus, J. L. Ternberg and R. E. Eakin (*J. Am. Chem. Soc.* **71,** *3858* (*1949*)) found that normal human gastric juice contained a nondialysable, heat-labile substance ("apoerythein") capable of combining apparently stoichiometrically with vitamin B_{12} to form a complex ("erythein") that was nondialysable and not dissociated by dialysis. Vitamin B_{12} bound in this fashion was unavailable to microorganisms. However, this complex could be dissociated by heat with liberation of vitamin B_{12} in a form again available as a microbiologic growth factor. The apoerythein in the gastric juice of anemic subjects appeared to be reduced in amount and the authors suggested,

without any supporting clinical evidence, that apoerythein probably was the "intrinsic factor of Castle or an important component thereof."

E. Beerstecher and S. Altgelt (*J. Biol. Chem.* **189,** *31* (*1951*)) accepted this terminology and interpretation, and found saliva from both normal subjects and patients with pernicious anemia to "bind" vitamin B_{12}. They concluded that the presence of hydrochloric acid in the stomach of the normal person is necessary to prevent destruction of salivary intrinsic factor. The numerous objections to the application of this thesis to pernicious anemia in the light of established clinical observations have been pointed out by A. D. Welch and R. W. Heinle (*Pharm. Rev.* **3,** *361* (*1951*)).

E. Beerstecher and E. J. Edmonds (*Fed. Proc.* **11,** *185* (*1952*)) have recently extended the complexity of these observations. They now describe apoerythein as present in gastric juice, and in gastric and duodenal mucosa as well as in saliva. Because of the presence of a heat-sensitized apoerythein inactivator in saliva, the thermal stability of salivary apoerythein differs from that of mucosal apoerythein. Gastric mucosal apoerythein is acid labile, thereby differing from gastric juice apoerythein. They also found that achlorhydric gastric juice contained an acid-labile apoerythein-inactivating enzyme. The lack of hydrochloric acid now appeared to them to be responsible for the absence of apoerythein or intrinsic factor from the gastric juice of patients with pernicious anemia. However, a simpler explanation for this would appear to be the insignificant volume of gastric secretion in pernicious anemia. Moreover, though lacking in hydrochloric acid, according to S. M. Goldhamer (*Am. J. Med. Sci.* **191,** *405* (*1936*)) such gastric juice may contain clinically effective concentrations of intrinsic factor when given in sufficient quantity.

In contrast to its inhibitory effect upon the availability of vitamin B_{12} for bacteria is the apparently facilitating effect of gastric juice for the utilization of vitamin B_{12} by bone marrow erythroid cells reported by L. G. Lajtha (*Clin. Sci.* **9,** *287* (*1950*)). He found, using the marrow of patients with pernicious anemia in tissue cultures made in diluted homologous serum, that vitamin B_{12} brought about no maturation of megaloblasts without the simultaneous addition of normal gastric juice (by itself inactive). It would seem reasonable to assume that since vitamin B_{12} is seemingly poorly absorbed by the mucosal cells of the gastrointestinal tract unless intrinsic factor is present, other cells as well might have difficulty in utilizing vitamin B_{12} unless a factor aiding absorption was present. However, Lajtha's experiments are difficult to reconcile with the *in vivo* observations of D. Horrigan, T. Jarrold, and R. W. Vilter (*J. Clin. Invest.* **30,** *31* (*1951*)) who reported that *local* maturation of megaloblasts was produced by instillation of pure vitamin B_{12} into the marrow cavity of patients with pernicious anemia.

The dissociating effect of heat upon the microbiologically inert complex of vitamin B_{12} plus "binding factors" has appeared to others to provide a means for study of the characteristics of such combinations with vitamin B_{12}. Although beef muscle was used as a source of extrinsic factor and hog stomach as intrinsic factor, L. Klein and J. F. Wilkinson (*Biochem. J.* **28,** *1684* (*1934*)) were the first to conclude that a substance of greater thermostability than the original hog stomach preparation resulted from incubation of the two substances at pH 5.7 and 37°C. for two or six hours. B. E. Hall *et al.* (*Proc. Staff Meetings Mayo Clinic* **25,** *105* (*1950*)) suggested on the basis of clinical responses in a patient with pernicious anemia that the complex formed between intrinsic factor (lyophilized duodenal mucosa) and vitamin B_{12} was more stable to heat than the intrinsic factor alone. F. H. Bethell, M. E. Swendseid, S. Miller, and A. A. Cintron-Rivera (*Ann. Int. Med.* **35,** *518* (*1951*)) noted a parallelism between the vitamin B_{12} "binding" property

and the intrinsic factor activity of human gastric juice and of extracts of hog stomach and duodenum. They reported that incubation with an extract of hog duodenal mucosa rendered the vitamin B_{12} microbiologically inert. The mixture was then treated with a trypsin preparation at pH 8 for several hours at 37°C. and heated at 90 to 100°C. for ten minutes to destroy any residual uncombined intrinsic factor. The resulting preparation produced an excellent therapeutic response when administered daily by mouth to a patient with pernicious anemia who had previously failed to respond to daily oral administration of vitamin B_{12} alone. Their conclusion was that "intrinsic factor when in combination with cobalamin is relatively heat stable and resistant to trypsin digestion."

On the basis of these and similar observations on the resistance of the unheated complex to microbiologic utilization, several workers have suggested that the role of intrinsic factor might be simply to make vitamin B_{12} unavailable for competitive utilization by intestinal bacteria. Thus, in its absence, the bacteria are presumed to take up vitamin B_{12} and the host to suffer from vitamin B_{12} deficiency. This explanation finds apparent support in the observations of H. Lichtman, V. Ginsberg, and J. Watson (*Proc. Soc. Exp. Biol. Med.* **74,** *884 (1950)*) who demonstrated hematopoietic effects of oral vitamin B_{12} in pernicious anemia when the intestinal flora was presumably inhibited by the oral administration of aureomycin. Moreover, B. von Bonsdorff in Finland has demonstrated the inhibitory effect upon hematopoiesis of the living broad tapeworm in anemic patients harboring this parasite when either vitamin B_{12} or beef muscle is given by mouth. Similar inhibition sometimes occurs even when gastric juice is also given. Von Bonsdorff and R. Gordin (*Acta med. Scandinav., Suppl.* **266,** *283 (1952)*) have attributed this to competitive assimilation of vitamin B_{12} by the parasite which they have shown actually to contain vitamin B_{12} in considerable amounts.

Although tempting because of its simplicity of demonstration *in vitro*, recent findings reported by G. H. Spray (*Biochem. J.* **50,** *587 (1952)*) do not support this interpretation of the physiologic action of intrinsic factor *in vivo*. This author first confirmed the results of Ternberg and Eakin (*loc. cit.*) by showing that incubation of vitamin B_{12} and acid gastric juice made vitamin B_{12} unavailable to *Lactobacillus leichmannii*, and that heating at 100°C. for five minutes made the vitamin again microbiologically active. If, however, the gastric juice was neutralized before vitamin B_{12} was added, prolonged heating or autoclaving was needed to release the vitamin from its combination and make it again available as a bacterial growth factor. Therefore, if the hypothesis concerning the role of intrinsic factor in protecting vitamin B_{12} from bacterial utilization is correct, it would appear that this particularly stable combination of vitamin B_{12} and intrinsic factor should be an effective oral antipernicious anemia preparation.

As a test of this possibility a number of samples of neutralized human gastric juice were pooled and a portion was incubated for twenty-two hours at 37°C. with vitamin B_{12} and then heated at 95°C. for twenty minutes. This preparation, although containing 5 micrograms of vitamin B_{12}, was found in microbiologic tests to be inert. It was also found to be hematopoietically inactive when administered by mouth daily to a patient with pernicious anemia in relapse. Thereafter the patient made a significant hematopoietic response to daily oral administration of 5 micrograms of vitamin B_{12} in neutralized gastric juice incubated for twenty-two hours at 37°C. but *not* then heated. This result, incidentally, would be anticipated from the previous observations of W. B. Castle, C. W. Heath, M. B. Strauss, and R. W. Heinle (*Am. J. Med. Sci.* **194,** *618 (1937)*) who showed that the intrinsic

factor of gastric juice either alone, or after incubation with beef muscle, is destroyed by heating at 100°C. for five minutes. At any rate, Spray's treatment of neutralized gastric juice which rendered the vitamin B_{12} unavailable to bacteria without destroying it did not potentiate its hematopoietic power when administered by mouth. This would suggest that intrinsic factor does not act primarily or entirely by protecting vitamin B_{12} from bacterial destruction. Indeed, this observation clearly demonstrates a difference in thermostability between intrinsic factor and the vitamin B_{12} binding factor.

Additional evidence against the physiologic significance of the vitamin B_{12} binding effect of sources of intrinsic factor was found by W. H. Prusoff *et al.* (*Abstracts. 118th meeting Am. Chem. Soc., Chicago, Sept. 1950, p. 27A*) who reported that portions of powdered stomach with the greatest intrinsic factor activity in human assays do not have the most capacity to bind vitamin B_{12}. B. E. Hall (*Brit. Med. J.* **2,** *585 (1950)*) also reported that two extracts of gastric mucosa of swine had little or no intrinsic factor activity when tested clinically, although they showed strong binding capacity for vitamin B_{12} when tested microbiologically. Hall (*Ibid.*) also found that egg-white lysozyme combined with vitamin B_{12} to render it microbiologically inert, but did not potentiate its hematopoietic effect on oral administration in pernicious anemia.

Further suggestions concerning the nature of intrinsic factor activity include the idea that folacin acts like intrinsic factor in promoting the utilization of orally administered vitamin B_{12} (L. M. Meyer, A. Sawitsky, N. D. Ritz, and M. Krim, *Am. J. Clin. Path.* **20,** *454 (1950)*). This interpretation is based on inadequate evidence since the possibility of a summation of therapeutic effects was not adequately excluded. C. C. Ungley (*Brit. Med. J.* **2,** *905 (1950)*) concluded that neither fresh milk nor a whey concentrate was an adequate substitute for intrinsic factor. The suggestion that aminopolypeptidase is identical with intrinsic factor (G. Ågren and J. Waldenström, *Acta med. Scandinav.* **119,** *167 (1944)*; *Ibid., Suppl.* **196,** *432 (1947)*) is no longer tenable. R. F. Schilling *et al.* (*J. Lab. Clin. Med.* **36,** *942 (1950)*) showed that aminopolypeptidase failed to potentiate vitamin B_{12} on daily oral administration and that neutralized gastric juice known to contain intrinsic factor did not contain aminopolypeptidase.

On the basis of extensive chemical and clinical studies on glandular mucoprotein G. B. Jerzy Glass, L. J. Boyd, M. A. Rubinstein, and C. S. Svigals (*Science* **115,** *101 (1952)*) have concluded that glandular mucoprotein is the main carrier of intrinsic factor activity. When 30 micrograms of vitamin B_{12} were given daily, significant hematopoietic effects required 50 to 75 mg. daily of the glandular mucoprotein, and with 10 to 20 micrograms of vitamin B_{12} daily as much as 150 to 200 mg. of mucoprotein were needed. However, in view of the observation of Welch and Heinle (*Pharm. Rev., loc. cit.*) that only 0.6 mg. of a relatively crude fraction of gastric juice constituted an effective daily complement of intrinsic factor for 5 micrograms of vitamin B_{12}, identification of glandular mucoprotein with intrinsic factor seems unlikely.

While the list of substances found *not* to be Castle's intrinsic factor continues to grow, the over-all physiologic role of intrinsic factor in aiding the absorption of vitamin B_{12} from the gastrointestinal tract seems firmly established by the clinical evidence cited in the first paragraph of this review. The stoichiometric relation between intrinsic factor and vitamin B_{12} noted by Ternberg and Eakin (*loc. cit.*) appears not to exist, at least under clinical conditions. Thus, Jerzy Glass *et al.* (*loc. cit.*), in testing the intrinsic factor activity of glandular mucoprotein found, as indicated above, a reciprocal relationship between the amount of this substance and the amount of vitamin

B_{12} required for a significant clinical effect upon hematopoiesis. Likewise, R. O. Wallerstein *et al.* (*Proc. Am. Soc. Clin. Invest., May 5, 1952, In press*) found that the hematopoietic effect of the daily oral administration of 1 microgram of vitamin B_{12} and 10 ml. of normal human gastric juice was increased in subsequent periods in the same patients when either the vitamin B_{12} was increased to 5 micrograms or the gastric juice to 25 or 50 ml. daily. Although under the clinical circumstances the essential relations of these substances are perhaps not subject to a quantitative analysis, the experimental results add no support to a stoichiometric relation. Indeed, this fact and the seeming physiologic irrelevance of the vitamin B_{12}-binding power of gastric juice may indicate that the sought-for significant action of intrinsic factor is upon the intestinal wall rather than upon the vitamin itself.

VITAMIN D AND CITRATE METABOLISM

A possible relationship between citrate metabolism and certain physiologic effects of vitamin D has been suspected since it was first demonstrated that the addition of citrate to rachitogenic diets resulted in the prevention or cure of rickets in rats (B. Hamilton and M. M. Dewar, *Am. J. Dis. Child.* **54,** *548* (*1937*); A. T. Shohl, *J. Nutrition* **14,** *69* (*1937*)). Although Shohl and A. M. Butler (*New Engl. J. Med.* **220,** *515* (*1939*)) subsequently reported similar beneficial effects in rachitic infants, definite studies of the relationship have not been reported. A recent contribution to this problem is a study by H. E. Harrison and H. C. Harrison (*Yale J. Biol. Med.* **24,** *273* (*1952*)).

These workers have investigated the changes in serum and urine citrate occurring in 10 rachitic infants after the administration of vitamin D. All patients studied had clinical, radiologic and biochemical evidence of rickets and had received little or no vitamin D prior to hospitalization. While receiving diets of cow's milk supplemented with ascorbic acid (plus cereals and vegetables in the case of older infants) serial fasting determinations of calcium, phosphorus, citrate and alkaline phosphatase in the blood serum were carried out before and after the intramuscular administration of 600,000 units of vitamin D_2. Repeated x-ray studies of the bones were made during the investigation for correlation with the biochemical changes. In addition to the serum studies, determinations of twenty-four-hour urine citrate excretion were performed on 3 infants.

Sixteen normal infants studied during the same period revealed an average serum citrate concentration of 2.5 (standard deviation ± 0.59) mg. per cent. In 21 determinations on untreated rachitic infants the average serum citrate was 1.5 (standard deviation ± 0.38) mg. per cent. These values were found to have a statistically significant difference. Following therapy, the rachitic patients demonstrated, in all instances, a rise in serum citrate to 2.5 mg. or more. The rate at which this increase occurred was variable, with five to twenty days being required for definite changes to occur. Healing of the bone changes as seen with x-ray was always accompanied by a rise in serum citrate. In several infants elevation of the citrate level above 3.5 mg. per cent was recorded after vitamin therapy and the authors felt that this might represent a temporary state of hypervitaminosis D, followed by a return of the citrate to normal values.

Studies of the correlation of serum calcium and phosphorus levels with serum citrate levels revealed that there was no apparent relationship prior to treatment. During recovery from rickets, however, the

increase in both calcium and phosphorus appeared to parallel roughly the increases in citrate levels. Unfortunately, no comment was made concerning changes in serum alkaline phosphatase, if such occurred.

Daily urine citrate excretion averaged 70 mg. in one patient and 83 mg. in a second patient before treatment. After therapy, however, these values rose immediately, reaching maximums of 162 and 155 mg. respectively, indicating markedly increased excretion of citrate simultaneous with the increases in serum citrate. In the third patient whose urine citrate was followed, the first dose of vitamin D was followed by little or no response in serum phosphorus and citrate. However, serum calcium and urine citrate rose, although there was no evidence of bone healing shown by x-ray studies. Accordingly, a second dose of 600,000 units of vitamin D_2 was given twenty-five days later, which was followed by serum citrate and phosphorus elevations and healing of the bone changes. Urine citrate, however, showed but little further rise after the repeated therapy.

These studies appear to demonstrate rather conclusively that serum citrate and urine citrate excretion are influenced by vitamin D. The lack of calcium and phosphorus correlation with citrate levels before therapy and the subsequent approximate correlation after vitamin D administration suggest that the changes in these are independently related to the effect of the vitamin rather than being interdependent. The authors are unable to explain the mechanism of the citrate alterations during vitamin D therapy, although a number of speculations are possible. Their investigation does, however, serve to emphasize the paucity of knowledge available concerning vitamin D and citrate metabolism in human beings. Further studies of citrate metabolism will be awaited with interest, in the hope that they may shed some light on the site of action of vitamin D and perhaps on the problem of resistant rickets.

SOIL FERTILITY INFLUENCES ON ANIMALS

It was recently pointed out (*Nutrition Reviews* **9,** *353 (1951)*) that soil fertility is important to human welfare mainly because of its influence on the level of production of crops for consumption by animals or by humans. Conflicting claims have appeared regarding the influence of soil fertility on the nutritional quality of crops or animal products, and the above review emphasized that these conflicts can be resolved only through carefully conducted long-term studies.

Such a study was initiated at the Michigan Agricultural Experiment Station in 1945 and is planned for continuation for at least a ten-year period. The study was designed to answer the following question: Does the fertility of the soil affect the nutritive value of the crops, the nutrition of the cow, the nutritive value of the milk, and ultimately, the health of the consumer? The research problem and the procedure were outlined in an earlier paper by S. T. Dexter *et al.* (*Mich. Agr. Exp. Station Quart. Bull.* **32,** *352 (1950)*).

Ten pairs of heifers (from cows bred to the same sire) were selected for the experiment. One of each pair received feeds grown on well fertilized soil and the other received feeds grown on the same type of soil which had been severely depleted and unproductive. The crops selected to serve as feed were those that would most likely grow satisfactorily on both the fertile and the depleted soil. Brome grass and timothy were grown as the hay component of the ration and soybeans were grown as a protein supplement. Corn, wheat, and oats were grown as the grain components of the

ration. Yields per acre on the depleted soil were only about one-half those from the fertilized soil.

Both groups of cows were managed identically, were maintained on the same plan of nutrition for full milk production, and consumed approximately the same amounts of digestible protein and total digestible nutrients. The heifer calves are being retained in the respective herds as replacement stock and provide a means of ascertaining the cumulative effects on later generations of the continued use of feeds grown on depleted as well as on fertile soil.

In a recent paper the essential amino acid content of colostrum and milk proteins from the animals in the two herds was reported (C. W. Duncan, G. I. Watson, K. M. Dunn, and R. E. Ely, *J. Dairy Sci.* **35,** *128 (1952)*). Colostrum and milk samples were collected over a two-year period and analyzed for total crude protein (nitrogen × 6.38), and the ten essential amino acids. Data were obtained from 6 to 8 cows from each herd and in some cases samples from the same cow were available through three lactation periods.

The essential amino acid content of the colostrum and milk proteins and also the protein content were not affected by the level of soil fertility. The values reported for the amino acid content of milk protein agreed well with those in the literature (K. A. Kuiken and P. B. Pearson, *J. Nutrition* **39,** *167 (1949)*). However, threonine consistently was found to be about 15 per cent higher in the milk protein near the end of the lactation period than at sixty days post partum, a finding which appears to be new. This was true in the animals receiving the feed grown on the depleted soil as well as in those receiving feed grown on the well fertilized soil.

The Michigan workers concluded that the amount and kinds of amino acids in milk proteins remain rather constant irrespective of the soil fertility conditions under which the cow's feed is grown. In the initial report the nutritive value of the milk from the herds was tested in feeding experiments with rats. No differences could be detected that were traceable to soil fertility. No data were given as to whether or not the soil treatment had any influence on the composition of the crops produced. The conclusion was drawn that, based on the presently available data accumulated over a five-year period, there is no justification for assuming that the level of soil fertility can influence the quality of cow's milk. This is in no way meant to imply that farmers should not use fertilizers. On the contrary, the economic advantages of improved crop yields from fertilization of depleted soils are usually tremendous.

The importance of the study initiated by the Michigan Station is obvious. Further reports from the study should be regarded with interest by nutritionists. It should be remembered, however, that the composition and nutritive value of milk is very difficult to alter. Marked deficiencies, such as calcium or phosphorus, bring about a decline in milk production and seriously affect the health of the cow, yet have little or no effect in modifying the composition or quality of the milk.

NERVOUS SYSTEM AND ANIMAL BEHAVIOR IN B-COMPLEX DEFICIENCIES: MAZE LEARNING

Experimental vitamin deficiencies studied in animals and in man, and investigations on the relation of vitamin deficiencies to specific mental diseases were reviewed by S. W. Hardwick (*in* G. W. T. H. Fleming, *Recent Progress in Psychiatry* **2,** *111 (1951)*). It is well established that dietary deficiencies of several components of the B complex

(thiamine, riboflavin, pyridoxine, pantothenic acid) result in degeneration of nerve tissue, demonstrable histologically, and in impairment of physiologic functions.

Profound alterations in the function and structure of the nervous system resulting from prolonged, severe thiamine deficiency have been demonstrated by biochemical and neuropathologic methods. H. E. Himwich ("Brain Metabolism and Cerebral Disorders," *see esp. p. 202 ff., Williams and Wilkins, Baltimore (1951)*), having summarized a large body of literature, considers it a well established fact that thiamine is a part of the enzyme system involved in the oxidation of pyruvic acid, one of the intermediary links in the metabolism of carbohydrate, the nervous tissues' chief food stuff. In cerebral tissues excised from avitaminotic animals the rate of glucose oxidation is reduced and pyruvic acid accumulates. Human beings suffering from thiamine deficiency exhibit impaired carbohydrate metabolism, and excess of pyruvic acid (hyperpyruvemia) and lactic acid. While addition of thiamine does not increase oxygen intake in cerebral tissues from normal animals possessing a fully functioning enzyme system, in tissue from avitaminotic animals it does raise oxygen consumption.

For reasons that are not fully clear, the disorders of carbohydrate metabolism are not equally serious throughout the nervous system. The severity and duration of thiamine deficiency, and possibly other factors, not yet identified experimentally, may determine importantly the "points of weakest resistance." At the present time it remains puzzling why in prolonged thiamine deprivation in man it is the (peripheral) neurologic changes that constitute striking clinical features of the deficiency while in acute deficiency the personality changes, neurasthenic in character, are prominent.

Where an autopsy is possible and when the deficiency has already resulted in structural damages, the site of disturbed metabolism may be identified by the morphologic end results. A correlation between clinical symptoms and degenerative changes of the nervous system have been observed both in man and in experimental animals. H. R. Street *et al.* (*Yale J. Biol. Med.* **13,** *293 (1940/41)*) observed degenerative changes in the peripheral nerves and in the posterior columns of the spinal cord, resulting from long-continued subminimal intakes of thiamine. R. L. Swank (*J. Exp. Med.* **71,** *683 (1940)*) investigated the correlation of clinical behavior and neuropathology in avian thiamine deficiency, and found that the characteristic leg weakness of thiamine-deficient pigeons is due to degeneration of the peripheral nerve fibers (sciatic nerve). J. F. Rinehart, M. Friedman, and L. D. Greenberg (*Tr. Am. Neurol. Assn., p. 174 (1946)*) reported on the neuropathology of thiamine-deficient rhesus monkeys. Surprisingly enough there was no histologic evidence of peripheral neuropathy, and no lesions were found in the spinal cord. In the brain degenerative lesions were found most frequently in the nuclei of some of the cranial nerves (oculomotor, vagus, less frequently the auditory-vestibular nerve), other nuclei in the midbrain, pons and medulla, and in the cerebellum.

In the intact organism we have to rely on neurophysiologic signs for localization of the predominant metabolic depression in the peripheral nerves and the spinal cord, the brain stem, cerebellum, or the cerebral hemispheres. A detailed study of the reflex activities in acutely thiamine-deficient cats was carried out by G. M. Everett (*Am. J. Physiol.* **141,** *439 (1944)*). It revealed impairments of righting in air, the depression of vestibulo-ocular reflexes, decreased light reflex (small pupillary constriction following a light stimulus), and cerebellum dysfunctions manifested in a staggering, uncoordinated gait. Spinal reflexes, such as the knee jerk or withdrawal of a limb from a painful stimulus, were considered essentially normal. Also, general observations

of behavior indicated little impairment of cortical function. The author noted that the cats came when called, avoided obstacles when walking about, and followed an object with their eyes. The integrity of the hopping and placing reactions, having localization in the cortex of the cerebrum, was also considered evidence of normal cortical function.

In the rhesus monkeys studied by J. F. Rinehart, L. D. Greenberg, and L. L. Ginzton (*Blood* **3,** *1453* (*1948*)) the removal of thiamine from the diet manifested itself in diminished food consumption and weight loss within about two weeks. Later the animals became apathetic, inactive and weak. This was followed by muscular incoordination, drooping of the upper eyelid indicating the paralysis of the third cranial (oculomotor) nerve, and tremors.

Such qualitative observations are useful, but they represent only the first stepping-stone in the scientific study of behavior. For a more precise description of simple functions (*e. g.*, auditory acuity), or of the more complex functions of the central nervous system involved in learned behavior, and for the study of relationship to such numerical parameters as duration and severity of thiamine deprivation, quantitative technics must be applied.

The early research on the vitamins of the B complex and animal behavior was focused on learning, and more specifically, on maze learning. The animals were trained to run in mazes of the alley-type with food, serving as incentive, placed at the end of the maze (*see, e.g.*, S. Maurer and L. S. Tsai, *J. Comp. Psychol.* **11,** *51* (*1930*); *J. Nutrition* **4,** *507* (*1931*)). It is known that the scores made by the animals in a maze are readily influenced by changes in motivation. It becomes especially difficult to evaluate the performance of different groups of animals when different foods are used as "rewards." Since loss of appetite is one of the early symptoms of B-complex (and specifically of thiamine) deficiency, differences in the nutritional value of the food might give rise to marked differences between the experimental and control animals even in the case of a complete absence of alteration in the learning capacity of the animals.

The criticism does not apply to such use of mazes as have been made by M. H. Marx (*J. Comp. Physiol. Psychol.* **41,** *364* (*1948*)), and others before him (*e.g.*, K. S. Bernhardt, *J. Comp. Psychol.* **17,** *123* (*1934*) and E. Poe, C. F. Poe, and K. F. Muenzinger, *Ibid.* **22,** *69* (*1936*); **23,** *59, 67* (*1937*)) in studies on thiamine intake and learning ability, in which escape from water, and not the finding of food, served as the motive for the performance.

Such a water-maze was used by J. R. Whitley, B. L. O'Dell, and A. G. Hogan (*J. Nutrition* **45,** *153* (*1951*)) as the technic for study of the integrity of the central nervous system in their experiment on folacin deficiency. The experimental animals, offspring of mothers placed at maturity on a ration deficient in folacin (about 5 micrograms of folacin per 100 g. of ration), were reared on the same experimental ration. They appeared normal, *i.e.*, did not show symptoms of hydrocephalus (increased light transmission through the cranium in newborn animals; enlarged cranium and loss of equilibrium in older animals) which was noted in some of the offspring of rats fed the experimental ration. The normal-appearing animals were weaned at 4 weeks of age and fed the experimental ration until they were used for the maze studies at 8 to 10 weeks of age. Animals from the stock colony paired with reference to age, weight and sex served as controls (Number = 78 pairs). Additional controls (Number = 18) were provided by animals maintained on the basal (experimental) ration supplemented by folacin. The rats were given five learning trials each day for twelve days (total of 60 trials). Completion of one run without error was accepted as the criterion of the mastery of the maze. On the average the animals on the stock ration required 14

trials to learn the maze, the supplemented animals 13 trials, and the experimental (folacin-deficient) animals 22 trials. The number of errors made before mastering the maze were 186, 216, and 288 respectively. No significant difference was demonstrated between the performance of the two types of control animals, while deficient animals showed statistically inferior maze-learning ability. The authors believe that the damage, demonstrable at near maturity, has occurred before birth and should be ascribed to the deficiency of folacin in the maternal diet, but they are aware of the fact that the available data do not provide an adequate basis for accepting such a hypothesis.

An interesting approach to the problem of animal intelligence is represented by Maier's test of "reasoning," requiring "putting together" elements of past experience in order to find the correct path to one of two feeding tables (N. R. F. Maier, "Reasoning in white rats," *Comp. Psychol. Monograph* **6,** *No. 3* (*1929*)). The test detects the effect of brain injuries resulting from partial cortical destruction (*J. Comp. Neurol.* **56,** *179* (*1932*)), and the growth of intelligence reflecting maturation of the central nervous system (*J. Comp. Psychol.* **13,** *1* (*1932*)). The author stated that the test score is not greatly influenced by minor variations in motivation and that where such differences occur it is possible to evaluate them. In a study concerned with the effect of a lactose-free diet on problem-solving behavior in rats, in which statistically significant differentiation has been obtained by Maier's reasoning tests, the preliminary use of the maze tests indicated that the variable factor of motivation reduced markedly the value of the performance scores as criteria of learning ability (Maier and J. U. Longhurst, *J. Comp. Physiol. Psychol.* **43,** *375* (*1950*)). Maier's technic, testing a complex learning function, has been applied by H. C. Hamilton and E. B. Maher (*J. Comp. Physiol. Psychol.* **40,** *463* (*1947*)) to the study of the effects of glutamic acid on the behavior of the white rat, but so far as is known, it has not been used for investigating the relationship between thiamine intake and animal intelligence.

COBALT IN RATS

The importance of cobalt in the nutrition of ruminants has long been known (*Nutrition Reviews* **4,** *227* (*1946*); **8,** *280* (*1950*); **9,** *155*, *243* (*1951*); **10,** *65*, *177* (*1952*); H. R. Marston, *Physiol. Rev.* **32,** *66* (*1952*)). Attempts to show a role for cobalt in the nutrition of the rat or other nonruminant species have consistently been unsuccessful. More recent studies by W. G. Jaffé (*Arch. Venezolanos de Nutricion* **2,** *19* (*1951*)) present evidence for a function of dietary cobalt in rats. In earlier studies by this author (*Z. f. Vitamin-, Hormon- u. Fermentforsch.* **2,** *493* (*1949*)) on the adequacy of all-vegetable diets for mice and rats, diets consisting primarily of soya flour and corn meal supplemented with a salt mixture and known vitamins were fed. Animals on these diets grew slowly, there was a high percentage of sterile females, a high mortality in the litters, and many females died during lactation. Vitamin B_{12} was not available for trial at that time.

In more recent studies similar diets were fed, consisting of defatted soya flour, corn meal, 5 per cent vegetable oil containing 0.2 per cent percomorph oil, 0.2 per cent wheat germ oil, 2 per cent salt mixture containing 0.1 per cent cobalt chloride, and all of the known vitamins of the B complex with the exception of vitamin B_{12}. Reproduction in mice for ten generations and in rats for six generations was good. In mice the number of animals weaned per litter was

essentially the same as that observed upon a stock diet. Growth in young animals was very slow after the first generation. Weaning weight was about 10 to 11 g., compared to 15 g. in control animals. Other than the slow growth, the only abnormality observed was a hyperplasia of the kidneys with a high ratio of kidney weight to body weight.

Rats behaved similarly. Mortality in the litters was greater than in the controls and growth was slower. These animals weighed 40 g. at 4 weeks of age compared to 70 g. for control animals. In either species, the injection of a single dose of vitamin B_{12} increased the weight of the young to essentially normal levels. It was clear, therefore, that although the diet was sufficiently low in vitamin B_{12} to produce a clear-cut deficiency in the litters, there appeared to be no effect upon reproduction and lactation over six to ten generations.

This finding was in marked contrast to the finding in the earlier work where the major difficulty appeared to be in the female animals during pregnancy and lactation, and in the high mortality of the young during lactation. A comparison of the two diets fed revealed that the only obvious difference was in the cobalt which had been added to the diet in the latter study. A new series of studies was undertaken to ascertain whether this was the factor involved. Both rats and mice were fed the above vegetable diets with or without cobalt additions. In mice without cobalt supplementation the following results were obtained: from 27 litters, 9 litters died and only 2.7 young per litter were weaned. When cobalt was added to the diet or to the drinking water, 4 litters out of 40 died and an average of 4.7 young per litter were weaned. Growth of the young was slow whether cobalt was added or not, as indicated by the average weaning weight of 10.5 g. without cobalt and 11.4 g. with cobalt. Injections of cobalt appeared to have no effect on the results.

Similar results were found with rats. Ten of 15 litters died on the basal diet and only 1 rat per litter was weaned. The average weight of these animals was 35 g. at weaning. With cobalt in the diet, 6 of 11 litters died, and 2.5 animals per litter were weaned, with an average weight of 45 g. With cobalt in the drinking water, 8 of 20 litters died, 4.3 rats per litter were weaned and these weighed on the average 64 g. The latter results approximate those obtained with vitamin B_{12} in the diet.

Additional studies showed that when the level of soya flour in the diet was raised from 25 per cent to 40 per cent the mortality of the young was higher and their growth rate was poorer than on the usual diet. Single injections of vitamin B_{12} were again adequate to overcome all signs of the deficiency.

These results apparently demonstrate that the function of cobalt in the rat and the mouse is similar to that in ruminants, but that these animals are much less able to utilize the cobalt in the diet. The simplest explanation for the results is, of course, that the dietary cobalt is utilized by the intestinal flora to make vitamin B_{12}. The author points out that no data are available upon the cobalt content of the basal diet and this would be expected to be of importance in the severity of the symptoms produced. With the additional cobalt supplied in these studies, there appears to be sufficient vitamin B_{12} made to meet the requirements for pregnancy and lactation, but generally not sufficient to allow normal growth in the young animals.

SUPPLEMENTATION TO THE RICE DIET OF SOUTH INDIA

The unraveling of the complex nutritional deficiencies seen in Southern India is a slow process. Much of a descriptive nature has been written, especially with respect to the

so-called "crazy-pavement skin," phrynoderma or "toad skin," and degeneration of the junction of the skin and mucous membranes affecting the lips, nostrils, eyelids, external auditory meatus, and the genital and anal regions (*Nutrition Reviews* **6,** *157 (1948)*). It has been stressed that these evidences of deficiency in India are probably not due to deficiency of a single dietary factor.

R. P. Devadas and T. S. Sutton (*Indian J. Med. Res.* **39,** *51 (1951)*) have studied the effect of the "poor rice diet" of South India on the growth of rats, and the results from supplementation with egg yolk, egg yolk extracts, casein, and riboflavin. The investigations were designed to show whether the fat-soluble or nonfat-soluble nutrients of egg yolk were effective to account for the known nutritive value of whole egg yolk in animals maintained on this rice diet.

Twenty-four-day-old rats with a low reserve of vitamin A in the liver (9 I.U.) were fed ad libitum and weights as well as food intake were recorded. The basal diet used was stated to be a modification of the "poor rice diet of South India" used in Madras, and was composed of 89 per cent converted rice, 3 per cent dried split peas, 3 per cent egg plant, 3 per cent unripe banana, 0.5 per cent each of sesame oil and mutton, and 1 per cent sodium chloride.

Rats supplied the basal rice diet gained very little weight during the first five weeks of the experiment. One group was then supplemented with riboflavin and the second with cod liver oil. Weight gains occurred in these two groups, but not in a third group where the diet was supplemented with crude casein. In contrast, the animals fed the basal rice diet with a supplement from the beginning of 30 per cent egg yolk gained weight very well and the addition of the above supplements did not increase the rate of weight gain.

When the egg yolk was partially extracted with ether its vitamin A content decreased somewhat, as did the rate of weight gain of the rats when the extracted material was used as a supplement. Progressively increasing extraction diminished the vitamin A content until none was present. Feeding the animals with the resulting material produced a progressive decrease in weight gain until finally growth was absent. Weight gain could be induced in the animals fed the highly extracted egg yolk by the addition of vitamin A. Riboflavin did not induce weight gain in these animals, but had an effect in addition to vitamin A when given with it.

Although vitamin A was absent in two of the most highly extracted egg yolk diets, the animals did much worse on that diet which was the most highly extracted (using ether, acetone, and alcohol). Because this effect did not seem to be due to any difference in vitamin A content of the diet, the authors conclude that some unidentified growth-promoting factor was present in egg yolk in addition to riboflavin and vitamin A. This conclusion is supported by the second paper by the same authors (*Indian J. Med. Res.* **39,** *59 (1951)*), in which it is shown that egg yolk extract added to the basal rice diet induced marked gains in weight. Moreover, the economy of food utilization (grams gain in weight per hundred calories of food consumed) also increased. This same effect was noticed when the basal diet with the addition of casein and lactalbumin was compared to that with egg yolk extract added. Here again there is an increase in weight gain and food consumption, and a marked increase in the economy of food utilization.

The studies were carried on for six weeks. When rats which were gaining weight satisfactorily with the egg yolk extract were given pyridoxine, calcium pantothenate and vitamin B_{12} after the fourth week, no increase in weight gain was observed. Apparently in spite of this, the animals looked

poorly, having "flabby skins, loose short hair, dirty tails, red-stained paws, whiskers, and abdomen." However, the addition of riboflavin to the diet did produce a further gain in weight, and deficiency symptoms were not noted in this last group of animals. The authors felt that egg yolk extract, because it produced these abnormal skin lesions and at the same time stimulated growth, accentuated a deficiency of riboflavin.

These studies are of course preliminary, since the number of rats used in each of the groups was relatively small (4 to 6 as a rule), and since some of the controls, with further refinement of nutrients undoubtedly planned for the studies, have not been reported.

REQUIREMENT FOR TRYPTOPHAN

That the science of nutrition is dynamic is demonstrated constantly as the results of research appear. Perhaps no field of interest exemplifies this thesis more than does the concept of the role of the amino acids; not only has the view regarding the relative significance of the so-called essential versus the nonessential amino acids altered within the past decade, but also the quantitative estimations regarding their nutritional requirements can be expected to change as the basal rations are improved and the growth rate of the experimental animals is consequently enhanced.

A case in point is the recent report on the requirement of tryptophan for growth (M. J. Oesterling and W. C. Rose, *J. Biol. Chem.* **196,** *33* (*1952*)). This amino acid, particularly, merits re-examination inasmuch as it is now known to be involved in the biosynthesis of niacin; the presence or absence of this vitamin, therefore, might conceivably alter the requirement of the test animal for tryptophan. For the foregoing reasons and because of other suggestive evidence (M. Womack and Rose, *Ibid.* **171,** *37* (*1947*); Rose, Oesterling, and Womack, *Ibid.* **176,** *753* (*1948*)) a re-evaluation of the relative growth-promoting activity of L- and D-tryptophan was likewise made.

The amino acid mixture of the basal diet contained no tryptophan; this amino acid was added in amounts of 0.1, 0.125, 0.15, 0.20 and 0.40 per cent in different assay experiments. Also, in one set of comparisons, niacin and liver extract were omitted, in another series liver extract only was given and in a third, both were present. The rats were so distributed that comparisons of growth on different concentrations of tryptophan were made with litter mates.

The data indicate that though no statistically valid difference could be demonstrated between growth on the 0.15 and 0.20 per cent levels of L-tryptophan, the tendency toward better growth on the diets with the higher concentration justifies its selection as a safe minimum requirement under these experimental conditions. This value has been tentatively suggested on the basis of earlier experiments (W. C. Rose, *Science* **86,** *298* (*1937*)). When DL-tryptophan was used at 0.20 per cent level, little if any difference in growth rate from that obtained with L-tryptophan at the same concentration was observed. At the 0.15 per cent level, however, there was a strong suggestion that the racemic mixture was somewhat inferior. When the comparison was made between the L-form and the D-form, the latter proved decidedly inferior at the 0.15 per cent level. It was calculated that roughly 75 per cent of the D-tryptophan consumed was rendered available for growth of the organism.

In all of the foregoing comparisons of the growth-promoting potency of D- and L-tryptophan, neither niacin nor liver extract

was present. In other comparisons it was observed that at the 0.15 and 0.20 per cent levels of tryptophan, nicotinic acid exerted no effect on the requirement for the L-form; at lower concentrations (which are subminimal), however, niacin enhanced the growth effect of the L-tryptophan. When, on the other hand, D-tryptophan was fed at the 0.15 per cent level with and without niacin, it was observed that the vitamin exerted a marked effect in augmenting the growth rate.

These newer studies serve to define more precisely the minimum requirement for L-tryptophan for the growth of weanling male white rats over a period of twenty-eight days and show that in the presence of the minimum requirement of this amino acid, niacin exerts no influence on growth. However, when suboptimal amounts of tryptophan are provided, the vitamin stimulates the growth of experimental animals. Comparisons made in this study show that D-tryptophan, contrary to earlier conclusions, is inferior to the natural form in promoting growth.

FLAVONOIDS AND X-IRRADIATION

Widespread hemorrhages are frequently observed in animals and human beings following acute whole body irradiation. An increase in capillary fragility has been suggested as one of the factors important in the pathogenesis of the hemorrhagic tendency in radiation injury. On the strength of this possibility, a number of investigators have studied the influence of flavonoids on the response to radiation. These investigations have been prompted by the supposed ability of the flavonoids to lessen hemorrhages associated with increased capillary fragility (*Nutrition Reviews* **2,** *309* (*1944*); **10,** *156* (*1952*)).

The data reported regarding the influence of the flavonoids on radiation injury are controversial. Some investigators have reported a marked reduction of mortality in flavonoid-treated animals; others have reported no change (*Nutrition Reviews* **7,** *317* (*1949*)). M. Dauer and J. M. Coon (*Proc. Soc. Exp. Biol. Med.* **79,** *702* (*1952*)) have recently reported on this problem.

Mice, rats, and guinea pigs were the test animals used in their studies. Various flavonoids given by different routes and at different times were used. The administration of flavonoids was begun seven to twenty-one days preirradiation and continued postirradiation until there were no survivors or until the thirtieth day postirradiation. Observations of the effects of the flavonoids on body weight and general appearance were also made in nonirradiated animals. Over 12 different flavonoids were used, including rutin in 3 different forms.

The radiation source used was a 250 kilovolt x-ray machine. The animals were exposed to the radiation in a revolving cage divided into concentrically arranged compartments. In most instances, a flavonoid-treated group of animals was irradiated simultaneously with an equal number of untreated controls. The dose rate varied from 31 to 50 roentgens per minute, and the total dose was administered at a single exposure.

Daily observations of body weights, deaths, and food and water ingested, if containing a flavonoid, were made. No observations were noted regarding the degree of bleeding after irradiation.

Data obtained in 1132 mice showed that neither rutin nor any of the other flavonoids administered exerted any influence on weight loss, time of onset of morbidity or mortality, or thirty-day mortality following exposure to 500 to 800 roentgens of total body x-irradiation. The LD_{50} based on the thirty-day mortality was approximately 550 roentgens.

Lack of protective influence of the flavonoids was also noted in 232 rats exposed to 500 to 700 roentgens of x-irradiation. Some of the flavonoid-treated animals were also given 100 mg. of sodium ascorbate subcutaneously during a seven-day post-irradiation period. No specific changes attributable to the ascorbic acid, or to the combination of ascorbic acid and flavonoids, were noted in the rats. J. B. Field and P. E. Rekers (*J. Clin. Invest.* **28,** *746* (*1949*); *Nutrition Reviews* **7,** *317* (*1949*)) previously found that the mortality in dogs exposed to x-irradiation was significantly reduced when ascorbic acid was given together with the flavonoid quercetin. No reduction in mortality occurred when the quercetin or ascorbic acid was given alone.

Observations of the effects of flavonoid treatment were also made in 70 young female guinea pigs. The results indicated that the various flavonoid preparations tested with or without vitamin C did not favorably influence the mortality consequent to a 250-roentgen single-dose total body x-irradiation.

In the study of Dauer and Coon, then, there was no evidence that the flavonoids offer any protection to mice, rats, or guinea pigs against the lethal effects of acute whole body x-irradiation. These findings are in keeping with the negative results reported in some earlier investigations, but contrary to the beneficial effects reported by others. Dauer and Coon point out some of the difficulties in interpreting the reported results; up to the time of their study, no two groups of workers had used the same combination of animal species, specific flavonoids, and route of administration of the agents studied. Beneficial results have been reported when certain flavonoids have been administered orally to dogs (Field and Rekers, *loc. cit.*), guinea pigs (W. G. Clark, R. P. Uncapher, and M. L. Jordan, *Science* **108,** *629* (*1948*)), and rats (B. Sokoloff, J. B. Redd, and R. Dutcher, *Ibid.* **112,** *112* (*1950*)). No beneficial effects were observed when the flavonoid was administered parenterally to mice (E. P. Cronkite *et al.*, *Proc. Soc. Exp. Biol. Med.* **70,** *125* (*1949*); Cronkite, W. H. Chapman, and F. W. Chambers, *Ibid.* **76,** *282* (*1951*)), rats (H. I. Kohn, P. W. Robinett, and M. N. Cupp, *Atomic Energy Commission Document No. 2176, Technical Information Division, Oak Ridge, Tenn.* (*1948*); H. M. Patt, M. N. Swift, and E. B. Tyree, *Atomic Energy Commission Document No. 2024-G, Technical Information Division, Oak Ridge, Tenn.* (*August 1, 1947*), and chicks (S. P. Stearner and E. J. Christian, *personal communication*), or orally to mice (H. S. Kaplan, *Quart. Report No. ANL-4205, 107, Argonne National Laboratory, Biol. and Med. Div., Chicago, Ill.* (*1948*)).

It should be mentioned that the premise on which the therapy of radiation injury with flavonoids is based, namely, an increase in capillary fragility as one of the important factors in the pathogenesis of the hemorrhagic manifestations, is a speculative one; no direct measurements of capillary strength in irradiated animals or patients have been published. In most studies, survival has been the principal criterion by which the prophylactic and therapeutic effects of the flavonoids have been judged.

It is apparent that at present no beneficial effects of flavonoids, including rutin, in radiation injury have been established.

BIOSYNTHESIS OF PROVITAMIN D_3 IN THE INTESTINAL WALL

Sterol compounds possessing vitamin D activity occur chiefly in animal tissues. They may also be produced by irradiation from precursors, or provitamins D. The important precursors are ergosterol (provitamin D_2) and 7-dehydrocholesterol (pro-

vitamin D_3). Ergosterol is found chiefly in plant sources, while 7-dehydrocholesterol is present in many animal tissues (*Nutrition Reviews* **5,** *35* (*1947*)).

Since vitamin D_3 would seem to be the principal compound with vitamin D activity occurring naturally in animals, the source of origin of its precursor, 7-dehydrocholesterol, is of interest. M. Glover, J. Glover, and R. A. Morton (*Biochem. J.* **51,** *1* (*1952*)) have concerned themselves with this problem. It seemed unlikely that certain of the dietary sterols, such as sitosterol, stigmasterol, and ergosterol, were compounds from which 7-dehydrocholesterol might readily be formed. The authors considered cholesterol as a more likely precursor. They have accumulated experimental evidence which indicates that 7-dehydrocholesterol is formed by enzymatic dehydrogenation of cholesterol, a process occurring most importantly in the intestinal wall.

A method for the determination of 7-dehydrocholesterol was developed and was utilized in these studies. The method takes advantage of the specific absorption of this sterol in ultraviolet light at a wavelength of 281.5 millimicrons. Determinations were done on the unsaponifiable fraction of tissue extracts. In certain instances, these extracts were freed of interfering materials by chromatographic separation of the sterol components.

First analyses were done in an effort to determine the relative concentrations of 7-dehydrocholesterol in a variety of tissues from guinea pigs and rats. In both species the concentration of the provitamin in the unsaponifiable fraction of tissues was greater in the small intestine than in any other of the organs analyzed. Provitamin D_3 had been regarded in the past as occurring chiefly in the skin, thus it is of interest that concentrations in intestinal tissues were three times those in the skin of rats. Mean concentrations were 0.45 per cent and 0.15 per cent of the total unsaponifiable fraction in small intestine and skin respectively. There was an even greater difference in concentration in these two tissues of guinea pigs. Of the unsaponifiable lipids of guinea pig intestine, 30 per cent was cholesterol and 6 per cent was 7-dehydrocholesterol. It was found that the duodenum contained larger quantities of the provitamin than did the upper and lower segments of the ileum. Most of the 7-dehydrocholesterol contained in the intestinal wall was found to be present in the mucosa. It was also found that the provitamin content of the small intestine in guinea pigs increased directly with increase in body size and age. Contents expressed as percentage of the total unsaponifiable fraction increased from 3.0 per cent in animals of average weight of 345 g. to 6.0 per cent in animals weighing 640 g.

When guinea pigs or rats were fasted or placed for two weeks on diets free of sterol, there was no significant reduction in the 7-dehydrocholesterol contained in tissues. The authors interpret the lack of decrease as an indication that the 7-dehydrocholesterol found in these tissues is not wholly accounted for by a dietary source of the provitamin.

Consideration was then given to possible enzymatic synthesis of 7-dehydrocholesterol from cholesterol. This hypothesis was tested by feeding 0.25 g. cholesterol to guinea pigs and then killing the animals at intervals up to nineteen hours. Determination of the 7-dehydrocholesterol content of the wall of the small intestine and the liver indicated that enzymatic dehydrogenation of cholesterol did occur. The quantity of the provitamin in the intestinal wall doubled in three to five hours after administration of the test dose of cholesterol and returned to predosage levels within eighteen hours. Similarly, the quantity of provitamin in the liver increased and remained at high levels for a somewhat longer period of time. The data indicated that the enzymatic reaction proceeded in the direction of an equilibrium with a ratio of 15:1 of cholesterol to 7-dehydrocholesterol. It should be pointed out

that only a very small fraction of the administered cholesterol could be accounted for as 7-dehydrocholesterol in the tissues of the small intestine and liver of these animals.

When the procedure was reversed and 0.2 g. 7-dehydrocholesterol were fed to guinea pigs, there was an increase in total steroid in both the intestinal wall and the liver, the degree of increase exceeding that which might be accounted for in terms of the increased concentration of 7-dehydrocholesterol in these tissues. This finding was taken as evidence that the enzymatic dehydrogenation of cholesterol is a reversible reaction and may move in either direction.

The authors carefully state that the methods utilized by them may not determine 7-dehydrocholesterol per se, but may include a group of compounds with the configuration of 7-dehydrosteroid. However, the data presented would indicate that most, if not all, of the absorption occurring in the region of the ultraviolet selected is with little doubt due to the specific compound under study.

These experiments provide evidence that a dietary source of provitamin D_3 may not be required and that the intact animal can synthesize 7-dehydrocholesterol from dietary cholesterol and presumably from cholesterol of endogenous origin. For synthesis of vitamin D_3, irradiation of the provitamin in the skin would be required. Glover and her associates (*loc. cit.*) found significant quantities of 7-dehydrocholesterol in the skin of guinea pigs, with none being contained in the hair or muscular layers of the skin. The intestine is apparently the chief site of synthesis of the provitamin. Whether 7-dehydrocholesterol has other roles in metabolism aside from its function as a provitamin cannot at this time be stated.

SYNTHESIS AND STORAGE OF PROTEIN

One of the concepts which might be said to characterize current thinking in the field of nutritional biochemistry and metabolism is that the tissue proteins (including those of the blood plasma) are not static, *i.e.*, chemically inert, but rather are in dynamic equilibrium with a nonprotein nitrogen pool. In other words, experimental evidence indicates that the tissue proteins are constantly being built up from simpler fragments or from amino acids and are also being degraded to simpler nitrogenous compounds. This concept views the living cell as a dynamic system in which are constantly taking place changes in protein as well as the evolution of energy from the other major foodstuffs.

Using data on the rate of excretion of orally administered labeled glycine, it has been possible by mathematical treatment of the observed values to calculate not only the rate of protein synthesis but also the magnitude of the nitrogen pool (D. B. Sprinson and D. Rittenberg, *J. Biol. Chem.* **180,** *715* (*1949*)). It was found that the rate of protein synthesis was 1.0 g. and 0.2 g. of nitrogen per day in the rat and in man respectively. The suggestion was also made that tissue proteins differ among themselves with respect to the extent to which they are involved in the dynamic equilibrium. The foregoing technical procedures and mathematical treatment of experimental data as well as the rate-process constants for protein synthesis and amino acid catabolism (H. D. Hoberman, *Yale J. Biol. Med.* **22,** *341* (*1950*)) have been applied in a study designed to determine the mechanism whereby nitrogen is stored in the body (P. D. Bartlett and O. H. Gaebler, *J. Biol. Chem.* **196,** *1* (*1952*)). Quantitative estimation of the rate of protein synthesis, of amino acid catabolism, and of the size of the nitrogen pool in animals in nitrogen balance on the one hand, and in animals in which has been

induced gain in body weight and in nitrogen storage, on the other hand, should give a clue to the chemical mechanism involved in growth and in nitrogen storage.

Adult dogs were brought to nitrogen balance; then 10 mg. per kilogram of body weight of N^{15}-labeled glycine was added to the diet, after which the urine was analyzed for N^{15} periodically for forty-eight hours. The same dog was then given anterior pituitary growth hormone parenterally; when the expected nitrogen storage had been induced for four days, the labeled glycine was again given and its excretion in the urine was measured. In these dogs the rate of protein synthesis per day was found to be 0.62 g. nitrogen per kilogram of body weight when the animals were in nitrogen balance. At this time the rate of amino acid catabolism and of synthesis of protein were the same. During induced growth with nitrogen storage, the size of the nitrogen pool (as grams of nitrogen per kilogram of body weight) was augmented by 44 per cent, the rate of amino acid catabolism was decreased by 42 to 67 per cent, and the rate of protein formation increased from 8 to 18.5 per cent. It appears, therefore, that the characteristic nitrogen storage of hormone-induced growth is the net result of a depressed rate of amino acid breakdown and of an augmented rate of protein synthesis.

There is ample experimental evidence to support the concept of a dynamic equilibrium between the proteins of the cell and those in the blood plasma. Detailed evidence supporting this view was secured from dogs which were maintained in nitrogen balance and given glycine labeled with N^{15} and in which growth was then induced by anterior pituitary growth hormone with the excretion of N^{15} again measured (P. D. Bartlett and O. H. Gaebler, *J. Biol. Chem.* **196,** *11 (1952)*). The plasma proteins were fractionated into (1) a globulin-rich portion, (2) an albumin-rich part and (3) a fraction containing $alpha_1$-acid glycoprotein and $alpha_2$- and beta-globulins of low molecular weight and peptides; the activity of the fractions was measured after administration of the labeled glycine.

The production of nitrogen storage by the growth hormone was accompanied by a higher total plasma protein concentration; fraction (3) showed a decrease, but the increase in fractions (1) and (2) accounted for the over-all increase in total protein. In both the nitrogen equilibrium and the nitrogen storage experiment, more N^{15} was incorporated in fraction (1) than in fraction (2). The slight decrease in concentration of fraction (3) in the growth experiment suggests that these proteins are utilized at a rate somewhat higher than that at which they are synthesized. Thus it appears that induced growth is reflected in an augmented rate of plasma protein formation, particularly in fractions (1) and (3), and it is shown again that these proteins are sensitive to the factors inducing general storage of protein in the organism.

SPECIFIC GRAVITY, SKINFOLDS AND BODY FAT

Morphologically and biochemically, the gross body weight is a very complex datum. The variable composition of the animal and human body makes difficult a proper interpretation of many nutritional experiments in which reliance has been placed on body weight.

In evaluating weight gain as a biologic criterion of the nutritive value of food proteins, H. H. Mitchell (*Indust. Eng. Chem.* (*Anal. Ed.*) **16,** *696 (1944)*) has stressed the point that gains in the body weight of growing animals may differ in their content of protein, fat, and water. Consequently, equal increments in the absolute weight do not necessarily represent equal nutritive effects

and may not be considered as biologically comparable. He noted that in research on farm animals it is generally recognized that live-weight increases do not represent a reliable measure of the nutritive effect. Evidence on the variable composition of live-weight gains has been obtained for rats and for chickens, and the tendency for the more rapid gains to have a greater content of fat and a smaller content of protein was demonstrated for cattle and for pigs.

It has been demonstrated, in small laboratory animals directly and in man indirectly (*Nutrition Reviews* **9,** *265* (*1951*)), that body weight may be separated into the fat fraction and the lean body mass on the basis of the specific gravity of the body. Data were needed which would strengthen the evidence for the validity of the method when applied to larger animals, closer to man than are the guinea pig (E. N. Rathbun and N. Pace, *J. Biol. Chem.* **158,** *667* (*1945*)) and the white rat (E. Da Costa and R. Clayton, *J. Nutrition* **41,** *597* (*1950*)). The data on specific gravity as a measure of the fat content of the pork carcass were reported by C. J. Brown, J. C. Hillier, and J. A. Whatley (*J. Animal Sci.* **10,** *97* (*1951*)). The 66 hogs were slaughtered at weights within a narrow range of 202 to 230 pounds. The animals represented inbred Duroc lines. They were quite fat, the ether extract accounting for 54.6 per cent of the body weight while the value for body water was very low (31.8 per cent moisture). The percentages were calculated on the basis of chilled carcass weight. The mean specific gravity was low (1.027).

It should be noted that the chemical analysis of the carcass was based not on the analysis of the whole body but on samples of four components (fat, the lean and fat "sausage" meat, bones, and skin). As the authors point out, errors in sampling the materials for chemical analysis, added to such technical sources of variation as the loss of moisture and fat in the process of cutting, grinding, and mixing, could account for the fairly low correlation between specific gravity and the "total fat."

Using the method of specific gravity, body composition in cattle was studied by H. F. Kraybill, H. L. Bitter, and O. G. Hankins (*J. Applied Physiol.* **4,** *575* (*1952*)). They wished primarily to check the validity of the method of estimating the amount of body fat from total body water, determined *in vivo* by the antipyrine dilution method (*Ibid.* **3,** *681* (*1951*)). Satisfactory *in vivo* measurements of specific gravity on mammals less cooperative than man are difficult —and even he may suffer from hydrophobia and claustrophobia when facing submersion! Air in the lungs and respiratory passages, air trapped in the hair, and the presence of gas in the abdominal viscera are potential sources of distressingly large errors. Kraybill *et al.* (*loc. cit.*) made their specific gravity determinations on eviscerated cattle. Body water was determined prior to slaughtering. The viscera were removed except for the kidneys, surrounded by fat (the perirenal fat depot), and their specific gravity was determined separately.

The specific gravity of 30 eviscerated yearling Hereford steers and heifers ranged from 1.017 to 1.070 (mean 1.045) while the physically separable body fat, calculated on the basis of the ninth to eleventh rib cut, ranged from 13.6 to 39.5 per cent of the body weight (mean 25.1 per cent). The relationship is an inverse one (the lower the specific gravity, the higher the "fat" content). The correlation was high. This indicates that the amount of adipose tissue in cattle can be calculated with considerable accuracy from specific gravity. The correlation between specific gravity and total body water was also very high. Simple equations were derived for estimating body fat and body water from the specific gravity values. The average water content of the lean body mass of cattle was given as 72.6 per cent. Knowing the total body water, the fat percentage may be calculated (fat per cent = 100 − (per cent water/0.726)).

Another technic, particularly valuable for field research, is the characterization of the individual's leanness-fatness on the basis of the thickness of the subcutaneous adipose tissue, the major factor contributing to the variability of the thickness of the skinfolds. Measurements of subcutaneous fat in man were carried out by the French anatomist, P. Richer, some sixty years ago (*Nouv. iconog. de la Salpêtrière* **3,** *20* (*1890*)).

For the study of modifications of the thickness of subcutaneous fat in different states of nutrition, V. Correnti devised an instrument referred to as a malachistometer (Greek malakos = soft, histos = tissue), an apparatus for the measurement of soft tissues (*Riv. di antropol.* **35,** *438* (*1947*)). The skinfold, lifted by an assistant, is placed between two flat, rectangular, metal jaws, one firm, the other operated by a micrometer screw. The instrument resembles an ordinary screw clamp, except that the pressure exerted by the jaw when the screw is being turned does not increase beyond a constant pressure. A click indicates when this pressure is reached.

Correnti applied the technic to 26 well nourished infants, 3 to 4 months old, and 21 severely malnourished infants and children, 3 to 30 months of age, of both sexes (*Arch. ital. pediat. e puericolt.* **14,** *119* (*1950*)). The skinfolds were measured at eight points. It is not so stated in the text, but the magnitude of the values leads one to believe that the author reported the findings as "skin" thickness (one-half of the skinfolds). Both the values for individual regions and a grand mean were given. The latter value for normal male infants was slightly smaller than for the females (0.507 versus 0.523 cm.); the mean value for the sexes combined is 0.512 cm. with a standard deviation of 0.085. The mean for the dystrophic children was markedly lower (0.223 cm.) and the relative order of the thickness at the different points showed less regularity. This fact should have been properly summarized in the form of a series of coefficients of correlation, but the number of cases was too small for a correlational analysis.

The relation between body build evaluated on the basis of ratings made from photographs and of body composition approached through specific gravity determinations was studied in a group of healthy men by C. W. Dupertuis and co-workers (*J. Applied Physiol.* **3,** *676* (*1951*)).

Their immediate interest in undertaking the study was much more mundane than are its broader implications. They wished to determine whether the ratings of the somatotype, with the three "components" of human physique (the round-soft = endomorphy, the square-muscular = mesomorphy, and the long-thin = ectomorphy), each rated on a scale from one to seven on the basis of photographs, could be used to predict specific gravity. The determination of the body volume by submersion is a fairly awkward procedure, inapplicable in most nutritional surveys and in many cases on the hospital ward. The result was at least partially positive. In groups with average specific gravity of 1.050, 1.066 and 1.084, corresponding to 24.3, 16.1 and 7.4 per cent of body fat, the endomorphy ratings decreased from 4.4 to 3.6 to 2.5.

This finding is of definite interest to the nutritionist and indicates the potential value of even a semiquantitative system of ratings, especially if it were accompanied by good instructions including photographic (and mensurational) standards, for a systematic evaluation of nutriture. Photographs have the advantage of a permanent record which can be remeasured and evaluated independently by different observers. Up to the present the tool has not been used to its full capacity. Technically, the absence of a workable and validated system of ratings and measurements has been perhaps the principal hindrance.

The external form of the body has fascinated medical men since Hippocrates' time, especially psychiatrists (*see* E. Kret-

schmer's book on body build and character, "Körperbau und Charakter," *20th edition, Springer, Berlin (1951)*) and internists, as well as novelists and poets, and the physical anthropologists in whose field the problem of description of body build professionally belongs. The most influential methodology in the study of body build was Sheldon's system of "somatotyping" (W. H. Sheldon, S. S. Stevens, and W. B. Tucker, "The Varieties of Human Physique," *Harpers, New York (1940)*).

The severe limitations of the "standard" (statistically normal) body weight are fully apparent. The standard weights incorporate the average increments in total body weight with age. In order to eliminate this factor in studying the age differences in body composition, a group of younger men (average age 21.8 years, height 175.0 cm., weight 73.5 kg.) was compared with an individually matched sample of older men (average age 50.3 years, height 175.3 cm., weight 73.3 kg.) of the same gross body size (J. Brozek and A. Keys, *J. Gerontol.* **6,** *Suppl. to No. 3, 67 (1951)*). The values of specific gravity were 1.073 and 1.058, indicating that 12.6 and 20.0 per cent of the body weight, respectively, are accounted for by "excess" fat. The indirect criteria (skinfold thickness, and differences between the circumference of chest and of abdomen) pointed in the same direction.

Additional data are needed to indicate the "standard" fat percentages at different ages. This would improve the procedure, introduced by R. A. McCance and E. M. Widdowson (*Proc. Roy. Soc. (London) B* **138,** *115 (1951)*), of classifying individuals as overweight or underweight (on the basis of computed or tabular standard weight for age and sex) and at the same time as lean or fat. The professional football players studied by W. C. Welham and A. R. Behnke (*J. Am. Med. Assn.* **118,** *498 (1942)*) would fall in the "overweight" but "underfat" quadrant.

The analysis of body composition in terms of gross tissue morphology constitutes a fairly recent, but attractive and promising approach to the evaluation of important facets of "nutritional status" as well as of other nutritional and, more generally, metabolic problems.

THE SOURCE OF SERUM ALKALINE PHOSPHATASE

The use of serum alkaline phosphatase levels as diagnostic indications has been widespread ever since H. D. Kay (*J. Biol. Chem.* **89,** *249 (1930)*) showed that phosphatase activity in the plasma was increased in various pathologic conditions. As this increase was particularly marked in bone diseases, Kay and other authors, in particular A. Bodansky and H. L. Jaffe (*Arch. Int. Med.* **54,** *88 (1934)*; *Am. J. Dis. Child.* **48,** *1268 (1934)*), have assumed that the additional serum phosphatase comes from the osseous tissues. In fact, more recently I. Motzok (*Biochem. J.* **47,** *193 (1950)*) showed that the increased serum alkaline phosphatase in rachitic chicks originates in the bone.

It is known, however, that a number of tissues contain appreciable levels of alkaline phosphatases. Besides bone, the kidneys, liver and intestine contain sufficient enzyme to be possible sources of the bulk of serum alkaline phosphatase. Indeed, it has been suggested that the small intestine (E. V. Flock and J. L. Bollman, *J. Biol. Chem.* **175,** *439 (1948)*) and the liver (M. J. Oppenheimer and Flock, *Am. J. Physiol.* **149,** *418 (1947)*; A. Cantarow and L. L. Miller, *Ibid.* **153,** *444 (1948)*) are the normal sources of serum alkaline phosphatase.

The fact that the activity of alkaline phosphatases characteristic of certain tissues is decreased differentially by a number of inhibitors has been used to differentiate

these enzymes. For instance, R. Cloetens (*Enzymologia* **7,** *157* (*1939*)) demonstrated that phosphatases are unequally affected by cyanide, and O. Bodansky (*J. Biol. Chem.* **118,** *341* (*1937*); **174,** *465* (*1948*)) showed that certain bile acids and amino acids also inhibit phosphatase preparations to varying degrees, depending on the tissue from which the latter are extracted.

A recent study (N. B. Madsen and J. Tuba, *J. Biol. Chem.* **195,** *741* (*1952*)), making use of five such inhibitors and of several physiologic settings, brings convincing evidence as to the main source of serum alkaline phosphatase in the rat. Madsen and Tuba studied normally fed untreated rats, fasted rats, animals receiving a 25 per cent fat diet, and alloxan-diabetic rats. The animals were killed by bleeding and the serum was separated immediately. Homogenates of kidney, liver, small intestine and bone (femur and tibia) were prepared. Phosphatase determinations were made on each tissue. The inhibiting effect of sodium taurocholate, cyanide, oxalate, L-glutamic acid and L-lysine on the phosphatase activity of each homogenate was measured. In many cases the effects of several dilutions of the inhibitors were determined. Besides the values corresponding to the uninhibited phosphatase preparations, Madsen and Tuba were thus able to present a total of 180 averages of 855 determinations, corresponding to a total of 9 dilutions of 5 inhibitors in four different experimental situations.

Both high-fat feeding and alloxan diabetes produced serum alkaline phosphatase levels very much higher than normal. In both cases, the inhibition pattern of serum phosphatase paralleled that of intestinal phosphatase. On the other hand, the phosphatases of other tissues showed inhibition patterns uncorrelated with that of serum. Madsen and Tuba concluded that the increase in serum alkaline phosphatase brought about by alloxan diabetes and by a high-fat diet originates in the intestine. Normal animals showed a lower serum alkaline phosphatase. Although the inhibition pattern did not resemble that of intestinal phosphatase so closely as in the two previous situations, the similarity appeared sufficient to justify the conclusion that the major portion of the normal serum alkaline phosphatase is also derived from the intestine.

In the case of the fasted rats, it was found that serum alkaline phosphatase fell to only 20 per cent of the normal level. The inhibition pattern of serum phosphatase in these animals was much less similar to that of intestinal phosphatase and appeared to indicate that bone contributed, in this case, an important fraction of the phosphatase activity. The authors indicate that work now in progress and following similar procedural technic demonstrates that bone is a probable source of most if not all of that portion of the serum phosphatase not supplied by the intestine, and confirm that the increased levels found in rickets are derived from osseous tissue.

The work of Madsen and Tuba thus brings additional evidence relative to the dependence of serum alkaline phosphatase on physiologic and dietary conditions, a conclusion also supported by other recent studies of serum alkaline phosphatase during food restriction and rehabilitation (E. Da Costa and R. Clayton, *Fed. Proc.* **11,** *439* (*1952*)). More importantly, it offers a convincing answer to the problem of the origin of serum alkaline phosphatase.

A NEW FORM OF VITAMIN B_{12}

Since vitamin B_{12} was isolated in 1948 (*Nutrition Reviews* **6,** *245* (*1948*)) a number of forms of the vitamin have been described (*Ibid.* **9,** *251* (*1951*)). The original form of the factor has been found to have an approximate formula of $C_{61-64}H_{86-92}N_{14}O_{13}PCo$.

The molecule has been fractionated by hydrolysis yielding, among other fragments, ribose, 5–6 dimethylbenzimidazole, 1-amino-2-propanol, and hydrogen cyanide. The cyanide is bound to the cobalt by one of the coordination links of the metal complex. Vitamin B_{12} can thus be described as a cyanocobalamine.

Two antipernicious anemia pigments, described separately as vitamin B_{12a} and vitamin B_{12b}, were later shown to be identical and to differ from vitamin B_{12} by the replacement of the cyano group by a hydroxyl group forming a hydroxycobalamine (E. A. Kaczka, D. E. Wolf, F. A. Kuehl, and K. Folkers, *Science* **112,** *354* (*1950*)). These workers also showed that a series of compounds, all retaining vitamin B_{12} activity, could be prepared by replacing the cyanide group by chloride, sulfide, or other groups susceptible of saturating the coordinate valences of cobalt. J. D. Buchanan, A. W. Johnson, J. A. Mills, and A. R. Todd (*Chem. Ind.* (*London*) *No. 22, 426* (*1950*)) isolated from *Streptomyces griseus* culture broths a more highly oxygenated form of the vitamin, which they called vitamin B_{12c}. All these forms showed the same biologic activity as vitamin B_{12} when animal growth was used as the yardstick.

There had been indications that one or more other vitamin B_{12}-like factors might be required for the growth of chicks. E. L. R. Stokstad and co-workers (*J. Biol. Chem.* **180,** *647* (*1949*)), and G. F. Combs and co-workers (*Ibid.* **182,** *727* (*1950*)), comparing the action of certain preparations with vitamin B_{12} had found that unidentified factors affected the growth of chicks relatively more than they did that of microorganisms and postulated that they represented new "animal protein factors." A vitamin B_{12}-like material (B_{12m}) had also been isolated from pig manure and was shown, by paper chromatography studies, to be different from any of the known forms of vitamin B_{12}. Nothing was known, however, of its growth-promoting ability. A report from the University of Wisconsin (U. J. Lewis, D. V. Tappan, and C. A. Elvejhem, *Ibid.* **194,** *539* (*1952*)) now brings conclusive evidence of the existence in the fecal matter of rats of a vitamin B_{12}-like material which not only differs from vitamin B_{12} in its chemical characteristics, but also presents different biologic properties.

Rats were fed a basal diet of which the main constituents were corn meal and soybean meal, with supplements of corn oil, salt, cystine, halibut liver oil, and crystalline vitamins. One group of animals received a supplement of 100 mg. of cobalt sulfate per kilogram of ration. Another group received daily injections of vitamin B_{12} (cobione). The vitamin B_{12} activity of tissues, urine, feces and gastrointestinal tract contents was determined by microbiologic assay using *Lactobacillus leichmannii*.

It was found that increasing the amount of inorganic cobalt in the diet caused no increase in the growth rate. By contrast, vitamin B_{12} administration was followed by a definite growth response. Inorganic cobalt also failed to change the vitamin B_{12} content of tissues, but it more than doubled the quantity of vitamin B_{12} of the feces. An increase in vitamin B_{12} activity was found along the entire length of the gastrointestinal tract, with the greatest concentration prevailing in the cecum. The possibility that the vitamin B_{12} thus produced, presumably by intestinal synthesis, was not utilized by the rat because it was formed too far down in the gut to be properly absorbed, was eliminated by careful rat-growth assays of the fecal matter. On the other hand, the capacity of this new material to promote the growth of young chicks was entirely comparable to that of vitamin B_{12}.

A study of this new fecal material was made by strip chromatography and by spectroscopy. By chromatography, the fecal material appeared not to be homogeneous; it was either contaminated with vitamin B_{12} or B_{12b}, or converted in part into these compounds by the analytic procedure. On

the other hand, repeated fractionation failed to change the characteristic absorption spectrum. Purification of the compound yielded crystalline red material. When this was dissolved in water, the solution was more orange than vitamin B_{12}.

The authors suggest that this new form of vitamin B_{12}, active in stimulating the growth of *L. leichmannii* and of the chick but presenting no rat-growth promoting activity, may be identical with one of the unidentified chick-growth factors previously postulated. While one could surmise a priori that this material may be an intermediate in vitamin B_{12} synthesis or an excretion form of the vitamin, Lewis and co-workers indicate that the new factor is probably a "biologically active compound in its own right." Because extremely high levels of vitamin B_{12} are needed to overcome the cobalt deficiency disease of sheep (D. E. Becker and S. E. Smith, *J. Nutrition* **43,** *87 (1951)*), the Wisconsin workers theorized that this new form of vitamin B_{12}, which appears to be synthesized by intestinal bacteria, might be the factor actually required by the sheep. In preliminary work they found that determination of the vitamin B_{12} content of sheep rumen material by microbiologic assay and by the rat-growth method gave values only one-third as high by the latter method as by the former. This discrepancy suggests that a large proportion of the rumen vitamin B_{12} might be similar to the new factor.

Such a possibility might give a clue to the understanding of some of the puzzling features of the cobalt deficiency anemia of cattle and sheep (*Nutrition Reviews* **9,** *243 (1951)*). In particular, it could explain why, if the "bush sickness" of cattle is essentially a vitamin B_{12} deficiency syndrome, and if the vitamin B_{12} required by these animals is identical to the human antipernicious anemia factor, neurologic manifestations have not been observed in cobalt-deficient cows and sheep (G. E. Cartwright, *Blood* **2,** *256 (1947)*). The discovery of a new vitamin B_{12} with different biologic properties from those of the classic forms may thus constitute an important step in the advance of our knowledge of hematopoietic processes and of the etiology of various forms of anemias.

GLUTAMIC ACID EXCRETION IN FOLACIN DEFICIENCY

The finding of an interesting additional relationship between folacin and glutamic acid, an amino acid which is an integral part of the folacin molecule, has been reported in two recent papers (H. A. Bakerman, M. Silverman, and F. S. Daft, *J. Biol. Chem.* **188,** *117 (1951)*); Silverman, R. C. Gardiner, and Bakerman, *Ibid.* **194,** *815 (1952)*).

The first report dealt with the influence of succinylsulfathiazole and folacin on glutamic acid excretion. Sulfathiazole derivatives are known to induce folacin deficiency (*Nutrition Reviews* **8,** *28 (1950)*). In an effort to determine the effect of dietary succinylsulfathiazole on the urinary excretion of amino acids by the rat, Bakerman and his co-workers analyzed the urine of rats administered the sulfa drug for fifteen amino acids. The experimental group was fed a synthetic diet containing 1 per cent of succinylsulfathiazole. Two groups of littermate controls were fed the unsupplemented diet, and the supplemented diet plus folacin, respectively. The amount of diet given the control group was limited to the intake of the experimental group. Portions of the collected urine specimens were autoclaved for the determination of "microbiologically available" amounts of amino acids. Other aliquots were hydrolyzed with acid for the determination of "total" amino acids. Concentrations of arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine,

leucine, lysine, methionine, phenylalanine, threonine, tryptophan, tyrosine and valine were measured.

Generally speaking, rats maintained on the basal diet plus succinylsulfathiazole excreted larger amounts of amino acids than did their litter-mate controls. These differences were small, however, except in the case of glutamic acid. Animals receiving succinylsulfathiazole in addition to the basal diet excreted from 50 to 2000 per cent (average, 1000 per cent) more "free" glutamic acid than did litter mates consuming the control diets. The presence of added folacin in the diet of the succinylsulfathiazole-supplemented control group maintained the excretion rate of glutamic acid at normal levels. Similarly, folacin treatment of rats which had become granulocytopenic on the succinylsulfathiazole-containing diet resulted in a return to normal not only of the leucocyte and granulocyte counts but also of the glutamic acid content of the urine.

Careful reconsideration of the steps involved in determining the urine content of the folacin (M. Silverman and co-workers, *loc. cit.*) showed, however, that the bulk of the microbiologically available or "free" glutamic acid contained in the urine of succinylsulfathiazole-treated rats arose during the heat sterilization process. During the treatment, a heat- and alkali-labile compound present in the urine was degraded to free glutamic acid. Although normally, when subjected to a temperature of 100°C. for an hour, neutralized urine specimens show a drop in glutamic acid activity because of the formation of a cyclic compound, specimens from folacin-deficient rats show an increase in glutamic acid under similar conditions. Conversion to glutamic acid of the hitherto unavailable derivative is maximal at a higher alkalinity. At a pH of 12, storage at room temperature suffices to induce the conversion. The compound can be retained on cation exchange resin and eluted with sulfuric acid. By a succession of such chromatographic procedures, an impure preparation of the compound was obtained. The molecular weight appears to be in the region of 500 and the molecule seems to contain two moles of glutamic acid and two atoms of nitrogen readily liberated in the form of ammonia.

The authors theorize that this derivative is perhaps a normal intermediate in the metabolism of glutamic acid and that a folacin-containing enzyme is required for its utilization. They are prompt to recognize, however, that any attempt to assess the significance of the occurrence of this glutamic acid derivative should properly await the completion of more thorough chemical work, namely, the determination of the composition and structure of this interesting compound.

URINARY EXCRETION OF 17-KETOSTEROIDS IN SCURVY

The close relationship between ascorbic acid and adrenal function has been repeatedly emphasized (*Nutrition Reviews* **3,** *37 (1945)*; **8,** *52 (1950)*). Adrenal tissue was one of the first materials from which ascorbic acid was isolated, and was soon after shown to be richer in ascorbic acid than any other animal tissue, with concentrations varying between 100 and 600 mg. per 100 g., depending on the source. A. Giroud, N. Santa, M. Martinet, and M. T. Bellon (*Compt. rend. Soc. de. biol.* **134,** *100 (1940)*) showed that, on scorbutic diets, the ascorbic acid and hormone content of the adrenal cortex fall simultaneously, the ascorbic acid level decreasing to 5 per cent of its original value while the hormone concentration is diminished by about two thirds. When ascorbic acid was injected, a rapid increase of this vitamin as well as of the

cortical hormone occurred, until normal levels were reached.

The classic experiments of Long and his collaborators (G. Sayers, M. A. Sayers, H. L. Lewis, and C. N. H. Long, *Proc. Soc. Exp. Biol. Med.* **55,** *238 (1944)*), demonstrating that injection of adrenotrophic hormone causes a fall in the ascorbic acid content of the adrenals, and observations of L. P. Dugal and M. Thérien (*see Nutrition Reviews* **6,** *143 (1948)*; **8,** *52 (1950)*) on the effect of ascorbic acid on resistance to cold, also demonstrated the closeness of the relationship between vitamin C and production of cortical hormones. An interesting paper by two Indian workers, S. Banerjee and C. Deb (*J. Biol. Chem.* **194,** *575 (1952)*), now brings evidence of a decrease in the urinary excretion of 17-ketosteroids in experimental scurvy.

It has been shown previously that the urinary excretion of 17-ketosteroids in females depends largely on the activity of the adrenal cortex. For example, the urinary excretion of 17-ketosteroids by women with Addison's disease is less than one third of the normal amount (R. W. Fraser *et al.*, *J. Clin. Endocrinol.* **1,** *234 (1941)*). Adrenalectomy also drastically reduces the 17-ketosteroid excretion of female monkeys (R. I. Dorfman *et al.*, *Endocrinology* **41,** *470 (1947)*). The authors accordingly compared the excretion of 17-ketosteroids by normal and scorbutic female guinea pigs. To eliminate the possible effect of inanition on steroid excretion, their animals were pair fed. From the second week of the experiment on, the guinea pigs fed a scorbutogenic diet started excreting progressively smaller amounts of 17-ketosteroids in the urine. By the fourth week, when the deficient animals exhibited definite scurvy, the amount of 17-ketosteroids excreted was about one third lower than that of their controls.

The study of Banerjee and Deb thus brings additional evidence for the existence of a state of hypofunction of the adrenal cortex in scurvy. The details of the relationships of cortical hormone synthesis with ascorbic acid remain one of the provocative problems of present-day biochemistry.

NOTES

Letter to the Editor

Dear Sir:

Your review on "Amino Acid Imbalance" which appeared in the May 1952 issue of *Nutrition Reviews* (**10,** *135* (*1952*)) was of great interest to us.

We would like to point out that interest in this problem began earlier than this review indicated. R. W. Jackson, B. E. Sommer, and W. C. Rose (*J. Biol. Chem.* **80,** *167* (*1928*)) and M. X. Sullivan, W. C. Hess, and W. H. Sebrell (*Pub. Health Reports* **47,** *75* (*1932*)) reported growth inhibition by glycine when this amino acid was added to a casein diet. Subsequently, we reported data showing growth inhibition resulting from amino acid imbalance (S. W. Hier, C. E. Graham, and D. Klein, *Proc. Soc. Exp. Biol. Med.* **56,** *187* (*1944*)). We concluded, "These data indicate the danger of imbalancing amino acid proportions by introducing high levels of a single amino acid even though it may be an essential amino acid."

Further data emphasizing the importance of the correct balance of amino acids in the diet of the rat were presented in our report before the American Chemical Society (*Abstract, 110th Meeting, Chicago, 7K,* (*1946*)), and again (C. E. Graham *et al.*, *J. Biol. Chem.* **185,** *97* (*1950*)).

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Composition of Foods Used in Far Eastern Countries

The second meeting of the Nutrition Committee for South and East Asia (*FAO Report, Washington, D. C.* (*1950*)) again emphasized the necessity for obtaining, in the near future, an adequate compilation of published and unpublished data on the composition of foodstuffs used in that region. No sound nutritional program and, therefore, no adequate agricultural planning could be established on insufficient knowledge of the nutrient content of the foods used in this region, inhabited by nearly half the human race.

A recent bulletin of the United States Department of Agriculture ("Composition of Foods Used in Far Eastern Countries," *Agriculture Handbook No. 34, U. S. Dept. of Agriculture, Washington, D. C.* (*1952*)) goes a long way toward bringing adequate solution to this problem. The authors, W.-T. W. Leung, R. K. Pecot, and B. K. Watt, have brought together data on food energy, composition with respect to water, protein, fat, total carbohydrate, fiber, and ash, as well as data on content of three minerals (calcium, phosphorus, iron) and five vitamins (vitamin A, thiamine, riboflavin, niacin, and ascorbic acid) and on refuse, obtained mainly from original studies conducted in the Far East and published in scientific journals. Three thundred and fortv-five references are given.

Particular attention was given to strict botanic identification and to the soundness of the analytic methods used. Correct use was made in the calculation of food energy of the Atwater method of specific coefficients; similarly, proper procedure was followed as regards calculation of protein content from results of nitrogen determination.

However, because of the few reported digestibility experiments effected with Far Eastern food, one is left to wonder whether the values of the specific Atwater coefficients are strictly applicable in this case. Similarly, some question arises as to the conditions in which "edible portions" and "as purchased" portions have been established. All workers who have had experience in the field of food composition and dietary surveys know the difficulties encountered in defining these terms and the great variability encountered in their use. When dealing with a region as diversified as the Far East, such differences

would appear to be considerable. While it is realized that, in establishing such tables, some reasonable average estimate must be arrived at, it would have been desirable to include in the introduction a short discussion of the principles followed in the main cases.

Dietary Fat and Multiple Sclerosis

Multiple sclerosis, a demyelinating disease of the nervous system, attacks spotty areas of the spinal cord so that the clinical manifestations are multiple and usually asymmetric. The etiology of the disease has been unknown, although anoxia, small thrombi, infections, and toxins, especially the heavy metals, have all been blamed. In addition, nutritional deficiencies, especially of B-complex vitamins, have been considered as possible causal conditions.

Another nutritional consideration, that of excess fat in the diet, has been suggested by R. L. Swank (*Am. J. Med. Sci.* **220,** *421 (1950)*). In this study the incidence of multiple sclerosis was correlated with the estimated dietary fat consumption in Norway, Denmark, Holland and Switzerland. There seemed to be some suggestive evidence of correlations, especially in Norway, where the incidence of this disease was known accurately between the years 1935 to 1948. During this time the estimated fat intake and the milk production paralleled the incidence of multiple sclerosis, rising in the years before World War I with the increase in milk production and fat intake, falling during World War II, and rising again after the end of the war. In Denmark and Holland the correlation was less remarkable. In Switzerland previous studies had suggested that multiple sclerosis was more common in the northern (German-speaking) parts of Switzerland than in the southern (Italian-speaking) cantons. Here again the evidence presented suggested that the fat content of the diet was very much higher in the German-speaking than in the Italian-speaking areas of this country. It was considered possible that the fat content of the diet was related to the incidence of the disease—not as the cause, but as a contributing factor.

A more detailed study of multiple sclerosis in relation to dietary fat intake has been made by R. L. Swank, O. Lerstad, A. Strøm, and J. Backer (*New Engl. J. Med.* **246,** *721 (1952)*). First an effort was made to learn the incidence of multiple sclerosis in all of Norway. Because of the general excellence of medical and diagnostic care throughout the country, it was considered that these data were quite reliable. In addition, occupation and other pertinent facts were learned from the patients.

Food consumption was studied in seven districts in the country by choosing a number of families (21 to 26 in each district) and determining the actual food consumption for the family for a period of one week. The intake per man per day was then calculated in terms of protein, carbohydrate and fat. Four earlier studies of similar nature were included. It was found that for the seven districts studied the intake of fat was high (from 148 to 179 g. per day in all) and did not vary greatly from one to the other. However, the farming communities consistently had a much higher butterfat intake than the fishing districts, whereas the opposite was true of the intake of margarine.

The authors considered that these data together with those of earlier studies suggested that a high intake of fat increased the incidence of multiple sclerosis by precipitating the disease in susceptible persons.

Swank and co-workers are careful to point out the possible fallacies and difficulties of a study such as this. Attempts were made to control some of the other environmental factors, but, of course, it was impossible to do so completely. The study is certainly not conclusive and will be difficult to substantiate or disprove unless it can be shown in experimental animals that a disease pathologically similar to multiple sclerosis can be induced by a high-fat diet.